

EXHIBIT 3

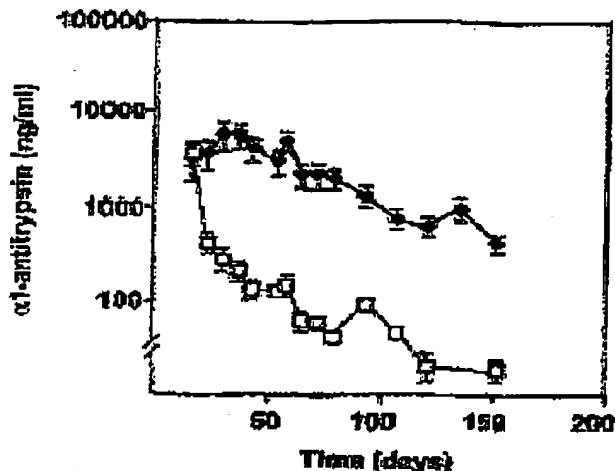


Figure Ad vector mediated coexpression of p21 enhances in vivo transgene expression delivered by a second Ad vector.

Mice were injected intravenously with mixtures of either 1×10^9 plaque forming units (pfu) of AdRSV-hAAT.2 and 1×10^9 pfu AdCMV.Null (open symbols) or 1×10^9 pfu AdRSV-hAAT.2 and 1×10^9 pfu AdCMV.p21 (filled symbols). After infection, blood was collected and serum levels of hAAT were determined by ELISA. Plotted are the mean \pm s.e. of individual measurements for each group.

This experiment demonstrates that p21 stabilizes the expression of a second gene not only in in-vitro cultured cells but also in vivo. Furthermore the data show that human p21 is also able to stabilize gene expression in cells and organs of non-human origin.

Figure Legends

Figure 1

Replication-deficient recombinant adenovirus vector induces apoptosis by uncoupling of S-phase and mitosis. The corresponding flow cytometry analysis of cell cycle distribution (A, B) and in situ detection of apoptosis by TUNEL-assay (C, D) are demonstrated for LoVo cells 48 h after Ad vector infection. Cells were mock infected (buffer control) (A, C) or infected with an Ad vector carrying alpha-1 antitrypsin (100 plaque forming units per cell) (B, D).

Figure 2

Overexpression of p21 prevents adenovirus-induced apoptosis. In situ detection of apoptosis in LoVo cells 48 h after infection. Cells were mock infected with buffer (A, D), infected with an Ad vector carrying the human alpha-1 antitrypsin at a dose of 100 plaque forming units per cell (B, E) or infected with an Ad vector coding for p21 (100 plaque forming units per cell) (C, F). Shown are representative photographs at a magnification of 200-fold (A to C) and 600-fold (D to F).

Figure 3

P21 protects against adenovirus-mediated apoptosis by prevention of a G2-like arrest. Demonstrated is the flow cytometry analysis of cell cycle distribution of LoVo cells after 48 h of infection with different doses of Ad vectors expressing either the cDNA of human alpha-1 antitrypsin (●) or the cDNA of p21 (○). Shown are the relative percentages of the cell populations which are in the G₀/G₁ or G₂/M phase of the cell cycle as well as the percentage of living cells in the whole population (negative in the propidium iodid staining; PI). The data represent the mean ± standard error of three experiments.

Add symbols.

FIGURE 1

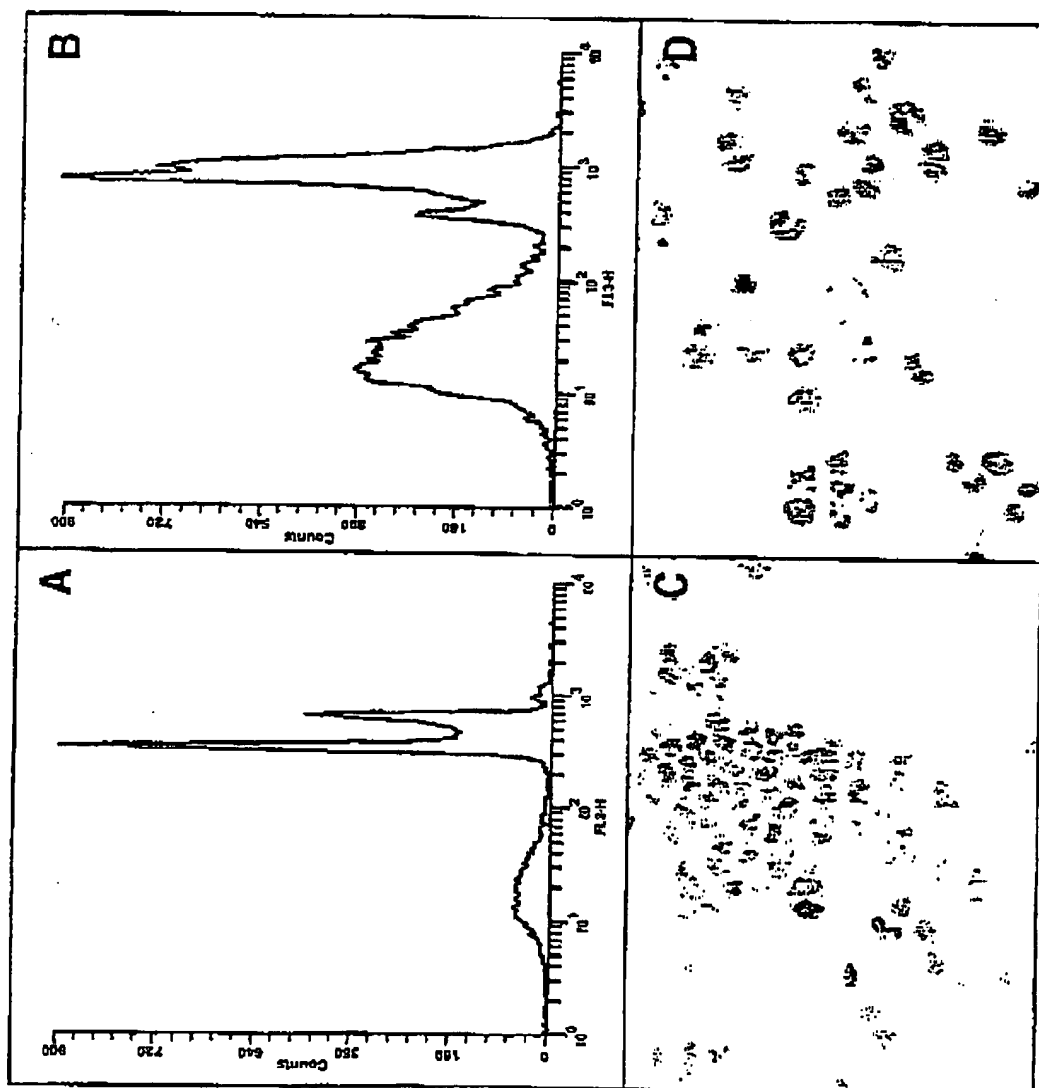


FIGURE 2

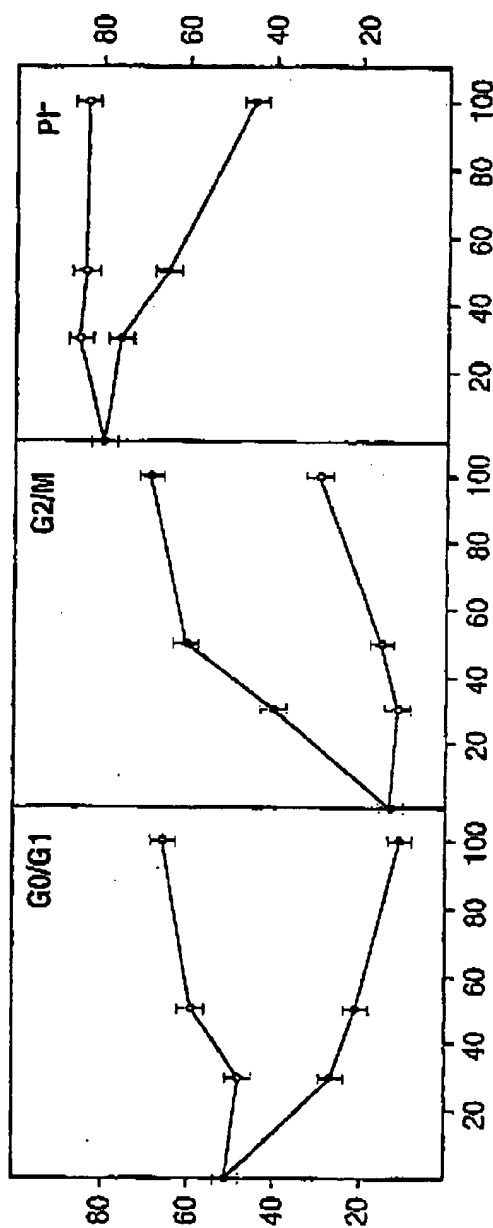


FIGURE 3

